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Linkage of Low-Density Lipoprotein Size to the Lipoprotein Lipase Gene in Heterozygous Lipoprotein Lipase Deficiency

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Summary

Small low-density lipoprotein (LDL) particles are a genetically influenced coronary disease risk factor. Lipoprotein lipase (LpL) is a rate-limiting enzyme in the formation of LDL particles. The current study examined genetic linkage of LDL particle size to the LpL gene in five families with structural mutations in the LpL gene. LDL particle size was smaller among the heterozygous subjects, compared with controls. Among heterozygous subjects, 44% were classified as affected by LDL subclass phenotype B, compared with 8% of normal family members. Plasma triglyceride levels were significantly higher, and high-density lipoprotein cholesterol (HDL-C) levels were lower, in heterozygous subjects, compared with normal subjects, after age and sex adjustment. A highly significant LOD score of 6.24 at $\theta = 0$ was obtained for linkage of LDL particle size to the LpL gene, after adjustment of LDL particle size for within-genotype variance resulting from triglyceride and HDL-C. Failure to adjust for this variance led to only a modest positive LOD score of 1.54 at $\theta = 0$. Classifying small LDL particles as a qualitative trait (LDL subclass phenotype B) provided only suggestive evidence for linkage to the LpL gene (LOD = 1.65 at $\theta = 0$). Thus, use of the quantitative trait adjusted for within-genotype variance, resulting from physiologic covariates, was crucial for detection of significant evidence of linkage in this study. These results indicate that heterozygous LpL deficiency may be one cause of small LDL particles and may provide a potential mechanism for the increase in coronary disease seen in heterozygous LpL deficiency. This study also demonstrates a successful strategy of genotypic specific adjustment of complex traits in mapping a quantitative trait locus.

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Introduction

A growing body of evidence supports the role of small, dense low-density lipoprotein (LDL) particles as a risk factor for coronary disease. Case-control studies have demonstrated consistently that a predominance of small, dense LDL particles is more common in individuals with coronary disease than in control subjects (Fisher 1983; Crouse et al. 1985; Austin et al. 1988*a*; Griffin et al. 1990; Tornvall et al. 1991; Campos et al. 1992; Coresh et al. 1993; Jaakkola et al. 1993; Griffin et al. 1994), and recent prospective studies indicate that the presence of small, dense LDL particles predict subsequent coronary disease events (Gardner et al. 1996; Stampfer et al. 1996; Lamarche et al. 1997). The association between small, dense LDL particles and coronary disease, however, may not be independent of other lipid factors. In particular, adjustment for plasma triglyceride (Tg) and high-density lipoprotein cholesterol (HDL-C) attenuates the magnitude of the association between small, dense LDL particles and coronary disease. A number of studies have shown a clustering of small, dense LDL particles, elevated Tg, and low HDL-C within individuals, leading to the proposal for an atherogenic lipoprotein phenotype (Austin et al. 1990*b*), a constellation of abnormalities that may indicate a common underlying cause leading to increased coronary-disease risk.

Studies have demonstrated both genetic and environmental influences on small, dense LDL particles. Complex segregation analysis in healthy families (Austin et al. 1988*b*; de Graaf et al. 1992; Austin et al. 1993*a*) and in families with familial combined hyperlipidemia (FCHL; Austin et al. 1990*a*; Bredie et al. 1996) provide evidence of a single major gene influencing small, dense LDL particles. In addition, these studies indicate that the expression of this complex trait is influenced by age, sex, menopausal status, and oral contraceptive use. Twin studies also indicate the presence of genetic and environmental influences on LDL particle distribution, with a range of 0.39–0.55 for heritability estimates (Lamon-Fava et al. 1991; Austin et al. 1993*b*). Genetic heterogeneity controlling LDL particle size is suggested by re-

ports that several loci may be linked genetically to LDL particle size. Nishina et al. (1992) reported linkage of small, dense LDL particles to markers for the LDL receptor (LDL-R) gene; however, no structural variants in the LDL-R gene were found in these families (Naggert et al. 1997). One sib-pair analysis has suggested multiple loci controlling LDL particle size (Rotter et al. 1996), including the LDL-R gene and the superoxide dismutase gene. Although linkage was suggestive for markers at the apo-C-III gene and the cholesterol ester transfer protein gene (Rotter et al. 1996), these values did not reach conventional thresholds for statistical support of genetic linkage (Morton 1955). A recent sib-pair analysis in female twins suggested linkage of LDL particle size to the apo-B gene but did not provide significant evidence for linkage of LDL particle size to the LDL-R gene or to the apo-C-III gene (Austin et al. 1998). At present, there is no definitive confirmation of the role of these genes in controlling LDL particle size.

Lipoprotein lipase (LpL) is an essential enzyme in the formation of LDL particles (Zambon et al. 1993). LpL hydrolyzes core Tg from very-low-density lipoproteins (Magill et al. 1982), a saturable process that leads to the formation of remnant lipoproteins, which then may be degraded by the liver or converted to LDL particles (Brunzell et al. 1973). Low levels of LpL activity are associated with an increase in LDL particle density (Hokanson et al. 1997*b*). More than 60 structural mutations in the LpL gene on chromosome 8p22, which abolish LpL activity and lead to LpL deficiency, have been identified (Brunzell 1995). Heterozygosity for these mutations is associated with low levels of LpL activity, elevated Tg, and low levels of high-density lipoprotein (HDL; Babirak et al. 1989; Emi et al. 1990; Wilson et al. 1990; Miesenbock et al. 1993). Preliminary data also indicate that LpL heterozygosity is associated with LDL particle density (Zambon et al. 1996) and small LDL particles, in one family (Miesenbock et al. 1993). In addition, there are structural mutations in the LpL gene that are associated with coronary disease (Hokanson 1997; Nordestgaard et al. 1997). These observations lead to the hypothesis that mutations in the LpL gene may be one cause of the small, dense LDL particle phenotype.

The purpose of the present study was to determine whether the LpL gene is a quantitative trait locus (QTL) responsible for LDL particle size variation in families with known structural mutations in the LpL gene. The approach used to determine this was the classic LOD score–linkage method. This report also compared the ability of the LOD score method to detect linkage for a qualitative trait (LDL subclass phenotype) versus a quantitative trait (LDL particle size), and evaluated the impact on linkage analysis of appropriate within-genotype adjustment for physiologic covariates.

Families and Methods

Family Selection

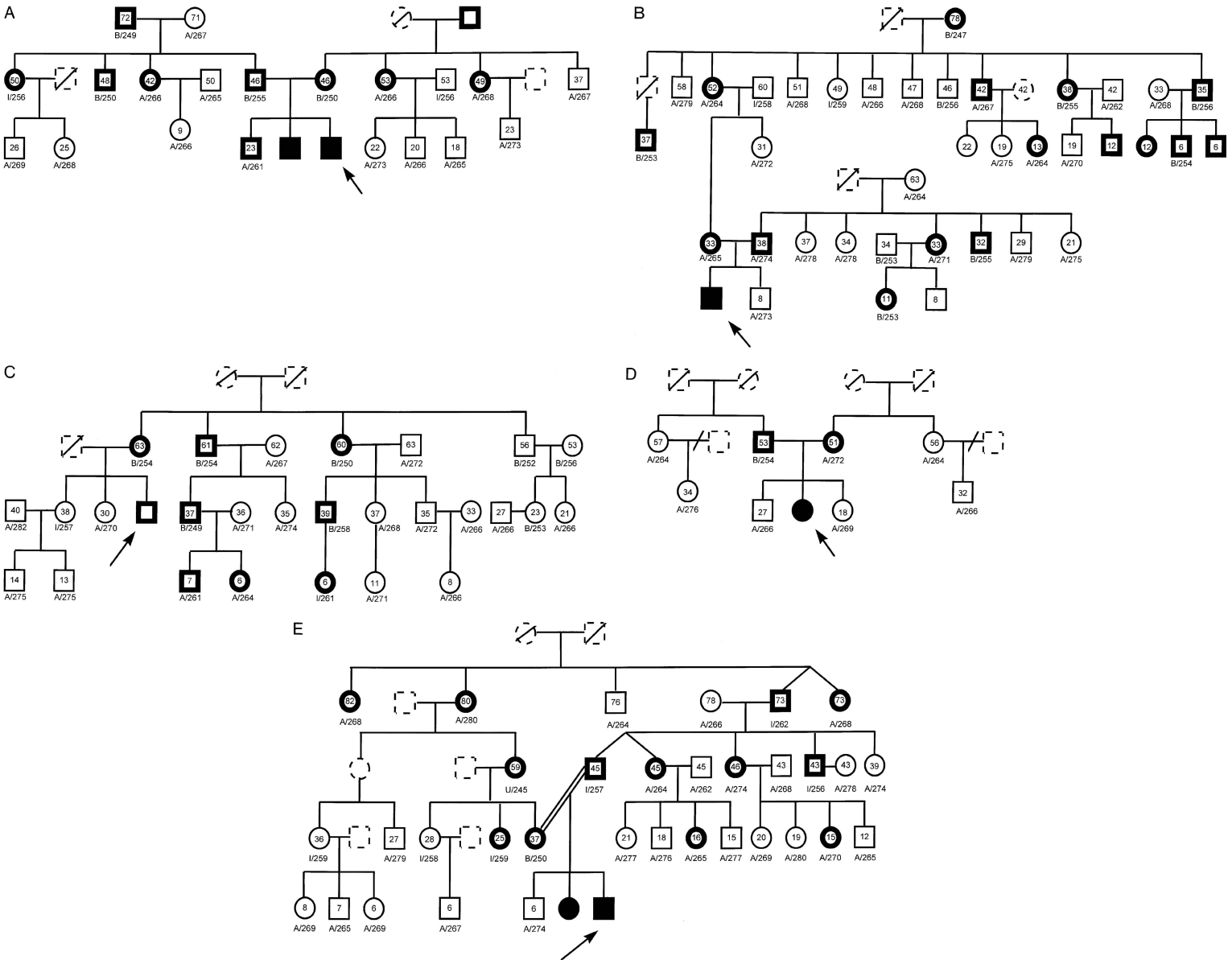
Families were identified through probands, referred for LpL deficiency, with a structural mutation in the LpL gene. Families were ascertained in this way because (1) the genetic markers used to genotype family members are functional mutations, not merely noncoding polymorphic markers; (2) the sample was as homogeneous as possible, with respect to LpL lipolytic function, thereby minimizing the necessary sample size; and (3) the information for linkage analysis to the LpL gene was maximized through sequential sampling on the basis of heterozygous genotypes.

Five extended kindreds were identified through probands referred for LpL deficiency, in which a primary defect in the LpL gene causing defective LpL lipolytic activity was identified (fig. 1). Four probands were referred as infants with chylomicronemia and failure to thrive (families 1, 2, 4, and 5), and the proband from family 3 was identified as a young man with severe hypertriglyceridemia. The presence of coronary disease in these families was not a criterion for selection. Probands, children <6 years of age, and pregnant women were excluded from the analysis. To maximize information for the linkage analysis, family members were recruited for this study on the basis of sequential sampling of first-degree relatives of individuals with an LpL mutation (Cannings and Thompson 1977). Of the 171 eligible subjects identified within these 5 families, 151 were contacted to participate in this study. We were unable to contact 20 potential subjects. Of the 151 potential participants, 21 did not meet the eligibility criteria and 10 refused to participate, resulting in a sample of 120 subjects for the analysis. The study design was approved by the University of Washington Human Subjects Committee, and all subjects provided written informed consent.

Laboratory Methods

Blood was collected in tubes containing 0.1% EDTA, from individuals who had fasted for 12 h. Each sample was centrifuged immediately, and plasma and white cells were kept at 4°C. Plasma was assayed for lipids and apo-B or was frozen at −70°C within 24 h of the blood draw. LDL particle size determination was performed on plasma frozen at −70°C within 24 h of the blood draw. Cells were stored at 4°C, prior to DNA extraction.

LDL particle size was determined by use of nondenaturing gradient-gel electrophoresis (Krauss and Burke 1982) with polyacrylamide gradients gels produced in our laboratory (Hokanson et al. 1997*a*). Whole plasma or LDL, isolated by sequential ultracentrifugation (Havel et al. 1955) and standards of known diameter, were


A
72 B/249
71 A/267
50 I/256
48 B/250
42 A/266
50 A/265
46 B/255
46 B/250
53 A/266
53 I/256
49 A/268
37 A/267
26 A/269
25 A/268
9 A/266
23 A/261
22 A/273
20 A/266
18 A/265
23 A/273
B
78 B/247
58 A/279
52 A/264
60 I/258
51 A/268
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48 A/266
47 A/268
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42 A/267
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38 B/255
42 A/262
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35 B/256
37 B/253
31 A/272
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19 A/275
13 A/264
19 A/270
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12
6 B/254
6
63 A/264
33 A/265
38 A/274
37 A/278
34 A/278
34 B/253
33 A/271
32 B/255
29 A/279
21 A/275
8 A/273
11 B/253
8
C
63 B/254
61 B/254
62 A/267
60 B/250
63 A/272
56 B/252
53 B/256
40 A/282
38 I/257
30 A/270
37 B/249
36 A/271
35 A/274
39 B/258
37 A/268
35 A/272
33 A/266
27 A/266
23 B/253
21 A/266
14 A/275
13 A/275
7 A/261
6 A/264
6 I/261
11 A/271
8 A/266
D
57 A/264
53 B/254
51 A/272
56 A/264
34 A/276
27 A/266
18 A/269
32 A/266
E
82 A/268
80 A/280
76 A/264
78 A/266
73 I/262
78 A/268
59 U/245
45 I/257
45 A/264
45 A/262
46 A/274
43 A/268
43 I/256
43 A/278
39 A/274
36 I/259
27 A/279
28 I/258
25 I/259
37 B/250
21 A/277
18 A/276
16 A/265
15 A/277
20 A/269
19 A/280
15 A/270
12 A/265
8 A/269
7 A/265
6 A/269
6 A/267
6 A/274

electrophoresed for 24 h at 125V (3,000 V-h), stained, and subjected to densitometry, and the diameter of the major peak of LDL was determined by use of a quadratic calibration curve of migration distance and particle diameters of the standards. LDL particle–size measurement is highly reproducible, with a coefficient of variation of 1.5% from 122 consecutive gels (Austin et al. 1995). LDL subclass phenotypes were defined as described elsewhere (Austin et al. 1988*a*; Austin et al. 1990*b*).

Cholesterol and Tg levels were measured by means of enzymatic methods, HDL was separated with use of dextran sulfate, and LDL cholesterol was calculated on the basis of the Friedewald equation (Capell et al. 1996). If Tg was >400 mg/dl, LDL cholesterol was calculated as the difference between the cholesterol in the $d < 1.006$ gm/ml fraction (Havel et al. 1955), minus the cholesterol in the dextran sulfate supernatant. The amount of apo-B was measured in plasma by immunonephelometry (Behring Diagnostic) with a polyclonal antibody (Marcovina et al. 1991). These measurements were performed at the Northwest Lipid Research Laboratory.

We extracted genomic DNA from leukocytes, after erythrocyte lysis, using 13.1 mM ammonium chloride and 0.9 mM ammonium bicarbonate (Poncz et al. 1982), by salt deproteinization (Miller et al. 1988), and resuspended it in 10 mM Tris-HCl, 0.2 mM Na_2EDTA (pH 7.5). The presence of variant LpL alleles was determined by means of primers and restriction enzymes, noted in table 1. Each gel contained a positive and negative control DNA. An LDL-R mutation (W66G) in family 2 was identified by use of dot-blot oligonucleotide hybridization (Leitersdorf et al. 1990).

Statistical Analysis

Analysis of differences in lipids and lipoproteins between heterozygous and normal family members used a robust estimator of variance to account for the lack of strictly independent observations resulting from genetic relationships, with the degrees of freedom determined on the basis of the number of families (i.e., five) (Lee et al. 1991; STATA statistical software). Regression analysis also used the reduced degrees of freedom, on the basis of clustered observations. All quantitative variables were adjusted linearly for age and sex, prior to analyses. There was no difference in the distribution of age or sex between LpL heterozygous and homozygous normal subjects (table 2); therefore, these adjustments were performed in the whole study population, irrespective of LpL genotype. Age and sex accounted for 9% of the variance in LDL particle size in this sample. Tg was transformed by the natural logarithm (ln).

Linkage analysis was performed by use of LOD score methods. We analyzed linkage of LDL subclass phenotype B (narrow definition; Austin et al. 1988*b*) to the LpL gene using model parameters, estimated from a prior segregation analysis, in Mormon families primarily (Austin et al. 1988*b*). There generally is less tobacco use and alcohol consumption among Mormons, and therefore there may be less confounding as a result of these factors in the segregation analysis. However, the segregation of LDL subclass phenotype B was similar in the one large non-Mormon kindred compared with the Mormon kindreds (Austin et al. 1988*b*). Although this segregation analysis could not exclude any specific single-gene mode of inheritance, the current linkage analysis used the model with a dominant mode of inheritance, done on the basis of maximum-likelihood estimation (i.e., a smaller -2 ln likelihood). The allele frequency was .25. Penetrance classes were determined on the basis of age, sex, and menopausal status. Specifically, the penetrance of phenotype B was estimated to be 17% for males $\leq$20 years of age, 82% for males >20 years of age, 21% for premenopausal women, and 95% for postmenopausal women (Austin et al. 1990*a*). Because medications that alter Tg levels may influence LDL subclass phenotypes (Austin et al. 1994), subjects taking fibric-acid derivatives ($n = 7$) were classified into the reduced-penetrance categories for their sex. Two subjects reported current use of oral contraceptives. Exclusion of subjects who use these medications did not alter the results (data not shown). The genetic marker for the LpL gene was diallelic (presence of any mutant allele vs. normal alleles). The allele frequency for the rare (mutant) allele was set at 10^{-3}, on the basis of the estimated frequency of homozygous LpL deficiency of $\sim 10^{-6}$ (Brunzell 1995).

Linkage analysis of LDL particle size (age and sex adjusted) as a continuous variable also was performed. LDL particle size also was adjusted for the potential effects of the LDL-R gene (Nishina et al. 1992; Rotter et al. 1996) in family 2, by use of linear regression.

Recent simulations from the Genetic Analysis Workshops have indicated the importance of appropriate ad-

Figure 1 Pedigrees of families with mutations in the LpL gene. *Panel A,* family 1. *Panel B,* family 2. *Panel C,* family 3. *Panel D,* family 4. *Panel E,* family 5. Arrows identify probands. Blackened symbols indicate LpL-deficient subjects. Note that all probands and LpL-deficient subjects were excluded from this analysis (see Families and Methods). Dark-bordered symbols indicate subjects heterozygous for mutations in the LpL gene. Light-bordered symbols indicate subjects homozygous for normal LpL alleles. Hatched-bordered symbols indicate subjects not sampled. Ages are indicated within the symbols. LDL subclass phenotype and LDL particle size (Å) are indicated below the symbol.

Table 1

Oligonucleotide Primers and Means of Detection for Specific Mutations in the LpL Gene

Exon	Mutation	Family Number	Primer Sequence (5′–3′)	Detection
3	W86R	4	AAGCTTGTGTCATCATCTTC ATAAGTCTCCTTCTCCCAGT	*Hpa*II digest
4	H136R	4	TTTTGGCAGAACTGTAAGCA GACAGTCTTTTCACCTCTTA	SSCP
5	G188E	1/5	TGTTCCTGCTTTTTTCCCTTT ATTAAGCGAAGATTTATTAT	*Ava*II digest
5	P207L	2/3	CATGCGAATGTCATACGAATGG TCCTGGCTGAAAAGTACCTCCACTC	*Dde*I digest
6	2-kb insertion	1	GCATGATGAAATAGGACTCC AATCTGACCAAGGATAGTGG	Oligonucleotide-specific primer

justment for covariates in mapping complex traits (Goddard et al. 1995; Wijsman and Amos 1997). Given the known relationship among LDL particle size, Tg, and HDL-C, linkage analysis was performed on LDL particle size also, after adjustment for Tg and HDL-C, by use of genotype-specific linear multivariate regression coefficients for ln, Tg, and HDL-C, in all families, simultaneously. This method of adjustment preserves the relationship of LDL particle size with Tg and HDL-C *between* heterozygous and normal genotypes and removes variance in LDL particle size related to Tg and HDL-C *within* genotypes.

Parameter estimates of the genetic model for LDL particle size were derived from a segregation analysis of LDL particle size (age and sex adjusted) as a continuous variable, reported elsewhere (Austin et al. 1993*a*), in the same primarily Mormon families as previously mentioned. Although no specific single-gene model could be excluded, the model chosen for the current linkage analysis was based on maximum-likelihood estimation. Allele frequencies were .18 and .82, with a dominant mode of inheritance for the rare allele, and genotypic means for LDL particle size of 258.7 Å and 268.5 Å, respectively, with a common variance of 6.8 Å for both genotypes. Genetic marker parameters were set to the same values as in the linkage analysis of LDL subclass phenotype B, as described previously. These parameters were used for all of the linkage analyses of LDL particle size as a continuous variable.

Linkage analyses were performed by the MLINK subroutine of the LINKAGE computer program, version 5.1 (Lathrop et al. 1984). Because this study specifically involved a linkage analysis of small LDL particles with a candidate gene (the LpL gene), LOD scores at recombination fraction (θ) of zero were of primary interest, although model misspecification might lead to a maximum LOD score (Z_{max}) at $\theta > 0$ (Clerget-Darpoux et al. 1986). We assessed heterogeneity of linkage between families using the admixture test (Smith 1963), by means of HOMOG, version 3.35 (Ott 1986).

Results

Five extended kindreds were identified, through probands referred for LpL deficiency, in which primary defects in the LpL gene causing defective LpL lipolytic activity were identified. Five different mutations in four exons of the LpL gene were identified among these five families (table 1). Mutations in probands of families 1 (G188E/2kb IN, exon 6), 2 (P207L), and 4 (W86R/H136R) were reported elsewhere (Brunzell 1995). Newly identified families with LpL mutations were families 3 (P207L) and 5 (G188E).

Of the 120 subjects, 46 were heterozygous for structural mutations in the LpL gene (table 2). Compared with homozygous normal subjects, heterozygous subjects had significantly higher Tg (264.2 ± 323 mg/dl vs. 123.4 ± 52.4 mg/dl, $P < .01$) and lower HDL-C (36.9 ± 11.1 mg/dl vs. 44.7 ± 11.2 mg/dl, $P < .02$). There was a trend toward higher levels of apo-B among heterozygous subjects, but the differences were not significant statistically. LDL particle size was significantly

Table 2

Sex, Age, Lipid, and Lipoprotein Values by LpL Genotype

Variable	LpL-Heterozygous Subjects	LpL-Homozygous Normal Subjects	P[a]
Sex (male/female)	20/26	36/38	...
Age (years)	40 ± 21	33 ± 18	>.10
Total cholesterol	203 ± 48.8	195 ± 55.8	>.50
HDL cholesterol	36.9 ± 11.1	44.7 ± 11.2	<.02
LDL cholesterol	117.2 ± 48.2	123.4 ± 52.4	>.20
Total triglyceride[b]	264.2 ± 323	139.3 ± 89.7	<.01
Apo-B	104.4 ± 44.9	86.9 ± 22.0	>.20
LDL particle size (Å)	259.6 ± 8.1	267.9 ± 7.0	<.005

NOTE.—Values are presented as mean ± standard deviation. All lipid and lipoprotein data are age and sex adjusted. Lipid and lipoprotein concentrations in mg/dl.

[a] Significance level estimated by robust standard errors and clustering analysis to account for potential nonindependence of familial data (see Statistical Analysis section).

[b] Tg was transformed with use of the ln value for statistical tests.

smaller among LpL heterozygous subjects compared with normal subjects (259.6 ± 8.1 Å vs. 267.9 ± 7.0 Å, $P < .005$). Among heterozygous subjects, the prevalence of LDL subclass phenotype B was 40%, versus 8% among homozygous normal subjects (fig. 2).

Within LpL-heterozygous subjects, there was a strong inverse relationship between LDL particle size and Tg levels (ln transformed, $r = 0.66$, $P < .005$), and there was a positive relationship between LDL particle size and HDL-C levels ($r = 0.57$, $P < .01$). A similar relationship was observed within homozygous normal subjects, with a significant inverse relationship between LDL particle size and ln-transformed Tg levels ($r = 0.73$, $P < .001$), and a significant positive relationship between LDL particle size and HDL-C levels ($r = 0.64$, $P < .01$). In multivariate analysis, both Tg and HDL-C levels were independent predictors of LDL particle size. Together, Tg and HDL-C accounted for 47% of the variance in LDL particle size, within heterozygous subjects, and for 58% of the variance within homozygous normal subjects.

Linkage analysis of LDL subclass phenotype B as a qualitative trait gave modest positive LOD scores but no statistically significant evidence of linkage (table 3; $Z_{max} = 1.65$ at $\theta = 0$, for all families). Among individual families, family 2 had $Z_{max} = 1.12$, whereas three families (families 1, 3, and 4) had only modest positive LOD scores. Family 5 had a small negative LOD score for linkage of LDL subclass phenotype B to the LpL gene, at $\theta = 0$.

Without adjustment of LDL particle size for the variance that results from Tg and HDL-C, there was suggestive evidence for linkage of LDL particle size as a quantitative trait to the LpL gene, with $Z_{max} = 2.95$ at $\theta = 0.11$. The LOD score at $\theta = 0$ was 1.54 (table 4). This may indicate a bias in the estimation of θ, as a result of model misspecification (Clerget-Darpoux et al. 1986). Four families had positive LOD scores, with a range of 0.26–1.50 at $\theta = 0$, whereas family 5 had LOD = −1.80. When LDL particle size was adjusted for Tg and HDL-C, irrespective of the subject's LpL genotype, there was no evidence for linkage of this trait to the LpL gene. The overall LOD score was −1.18 at $\theta = 0$ (table 5).

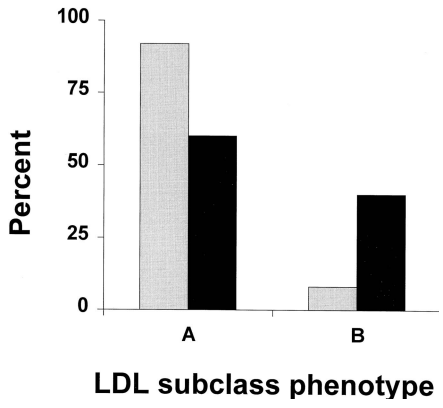

Figure 2 Prevalence of LDL subclass phenotypes. Light bars indicate subjects homozygous for normal LpL alleles. Dark bars indicate subjects heterozygous for mutations in the LpL gene.

Table 3

LOD Scores for Linkage Analysis of LDL Subclass Phenotype B to the LpL Gene

Family Number	LOD Score at θ =					
	.0	.1	.2	.3	.4	.5
1 ($n = 20$)	.30	.20	.13	.07	.03	.0
2 ($n = 32$)	1.12	1.00	.78	.48	.18	.0
3 ($n = 27$)	.42	.33	.21	.10	.02	.0
4 ($n = 8$)	.06	.03	.02	.01	.0	.0
5 ($n = 33$)	−.24	−.19	−.14	−.10	−.05	.0
Total	1.65	1.37	1.00	.56	.18	.0

Note.—Results were determined on the basis of five extended kindreds with LpL mutations.

Linkage analysis of LDL particle size, by use of the within-genotype adjustment for Tg and HDL-C, indicated strong evidence for linkage to the LpL gene (table 6). For all families combined, $Z_{max} = 6.24$ at $\theta = 0$, and the LOD score maximized at $\theta = 0$, in all families. Two individual families had LOD > 2 (family 2, LOD = 2.96; family 3, LOD = 2.18). Two other families showed modest positive LOD scores (families 1 and 5), and family 4 provided little additional information. The χ^2 test of heterogeneity gave no evidence of linkage heterogeneity among these families ($\chi^2 = 0.0$). Furthermore, LOD scores maximized at $\theta = 0$ (fig. 3), suggesting that the LpL gene itself, and not another gene in this region of chromosome 8p22, affects LDL particle size. Thus, these families provide statistically significant evidence of cosegregation of LDL particle size with the LpL gene.

Discussion

In the present study, we present strong evidence that mutations in the LpL gene affect LDL particle particle size. Linkage analysis of LDL particle size (after adjustment for within-genotype variance resulting from Tg and HDL-C) to the LpL gene produced highly significant evidence of linkage (LOD = 6.24 at $\theta = 0$). The strategy used in the analysis of this complex trait was to select families in which structural mutations in the LpL gene were known to be segregating, thereby obtaining maximally informative marker genotypes in the linkage analysis. In addition, the trait was refined by within-genotype quantitative adjustment for covariates (Tg and HDL-C)

Table 4

LOD Scores for Linkage Analysis of LDL Particle Size to the LpL Gene

Family Number	LOD Score at θ =					
	.0	.1	.2	.3	.4	.5
1 (n = 20)	.81	.62	.45	.29	.13	.0
2 (n = 32)	.77	1.67	1.54	1.10	.50	.0
3 (n = 27)	1.50	1.32	1.03	.67	.28	.0
4 (n = 8)	.26	.17	.09	.04	.01	.0
5 (n = 33)	−1.80	−.84	−.43	−.20	−.06	.0
Total	1.54	2.94	2.69	1.90	.86	.0

NOTE.—LOD score values reflect LDL particle size as a quantitative trait, adjusted for age and sex only, on the basis of five extended kindreds with LpL mutations.

that are associated with LDL particle size. The present study is the first to show evidence of genetic linkage of LDL particle size to a QTL with structural mutations that are known to influence protein function.

Although other studies have investigated linkage of LDL subclass phenotypes or LDL particle size to other candidate genes, the results have not been definitive. Nishina et al. (1992) reported evidence for linkage of LDL subclass phenotype B to the LDL-R gene, but they were unable to identify any mutations in the coding region of LDL-R gene in those families (Nishina et al. 1992; Naggert et al. 1997). One sib-pair analysis suggested that four genes influence LDL particle size, including the LDL-R gene (Rotter et al. 1996); however, in another sib-pair analysis, this was not confirmed (Austin et al. 1998). The present study does not exclude the possibility that the LDL-R gene influences LDL particle size. In fact, in one family in which the LDL-R structural mutation W66G was segregating (family 2), there was a modest 3-Å increase in LDL particle size among carriers with that LDL-R mutation, accounting for 4% of the variation in LDL particle size.

In another sib-pair analysis of polymorphic sites in and near the LpL gene, there was no evidence of linkage of the LpL gene to LDL particle size in families with coronary disease (Rotter et al. 1996). The apparent discrepancy with the present study may simply reflect the expected infrequency of mutations in the LpL gene in a sample not ascertained on the basis of these mutations. A second possible explanation is that sib-pair methods have low power relative to classic LOD score methods, if the genetic model is specified appropriately (Demenais and Amos 1989); thus, failure of the sib-pair analysis to detect linkage may represent a type-II error.

There are several important methodological aspects, which contributed to the ability to detect linkage in this study, that should be emphasized. The identification of families through probands with known mutations that alter protein structure and function provided a unique opportunity to assess the impact of the LpL gene on LDL particle size, because there is a direct relationship between the alleles of interest and altered LpL lipolytic function. This strategy led to a more genetically homogeneous sample, with respect to the LpL function. Use of such a homogeneous sample increases power in mapping a QTL for a complex trait.

In addition, these results clearly demonstrated the value of using quantitative traits, rather than qualitative traits, for linkage analysis. This recently was identified as a key factor in improving power in linkage analyses of simulated data (Graham et al. 1997; Wijsman and Amos 1997). In the present study, linkage analysis of the quantitative trait, LDL particle size, achieved a 78% increase in the Z_{max} compared with the linkage analysis of the qualitative trait, LDL subclass phenotype (table 3 and table 4). This illustrates the inherent loss of information that results from dichotomizing a quantitative trait (Graham et al. 1997). There is also loss of power resulting from the inability to adjust the dichotomous trait for the within-genotype effects of Tg and HDL-C.

Appropriate adjustment for covariates that influence LDL particle size was another important factor in identifying significant evidence for genetic linkage. When the within-genotype variance of LDL particle size related to Tg and HDL-C was taken into account, Z_{max} was more than twice that obtained without adjustment for these physiologic covariates (table 4 and table 6) and was more than sixfold higher when compared with the LOD score of LDL particle size adjusted for Tg and HDL-C, irrespective of genotype (table 5 and table 6). Thus, the adjustment of LDL particle size for the genotypic-specific effects of Tg and HDL-C illustrates the importance of appropriate covariate adjustment in detecting evidence for linkage of LDL particle size to the LpL gene. Similar results obtained on the basis of simulated data have been reported (Goddard et al. 1995; Wijsman and Amos 1997). The present study shows the striking impact of appropriate adjustment of physiologic covariates on mapping QTLs in a natural setting.

Small LDL particles, elevated levels of Tg, and low

Table 5

LOD Scores for Linkage Analysis of LDL Particle Size to the LpL Gene

Family Number	LOD Score at θ =					
	.0	.1	.2	.3	.4	.5
1 (n = 20)	−.65	−.36	−.19	−.08	−.02	.0
2 (n = 32)	−.02	−.01	−.01	.0	.0	.0
3 (n = 27)	−.43	−.21	−.11	−.06	−.02	.0
4 (n = 8)	.06	.03	.01	.0	.0	.0
5 (n = 33)	−.15	.04	.08	.06	.03	.0
Total	−1.18	−.51	−.21	−.08	−.01	.0

NOTE.—LOD score values are adjusted for age, sex, and variance resulting from Tg and HDL-C, irrespective of genotype, in five extended kindreds with LpL mutations.

Table 6

LOD Scores for Linkage Analysis of LDL Particle Size to the LpL Gene

Family Number	LOD Score at θ =					
	.0	.1	.2	.3	.4	.5
1 (n = 20)	.67	.48	.29	.14	.04	.0
2 (n = 32)	2.96	2.92	2.34	1.54	.67	.0
3 (n = 27)	2.18	1.73	1.26	.76	.29	.0
4 (n = 8)	.03	.02	.01	.0	.0	.0
5 (n = 33)	.41	.33	.16	.04	.01	.0
Total	6.24	5.48	4.00	2.49	1.01	.0

NOTE.—LOD score values are adjusted for age, sex, and within-genotype variance resulting from Tg and HDL-C, in five extended kindreds with LpL mutations.

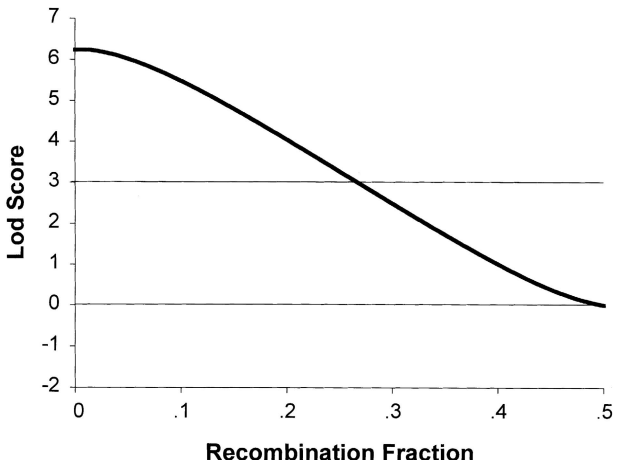

Figure 3 LOD scores at θ = 0–0.5, for linkage of LDL particle size to the LpL gene, after adjustment for within-genotype effects of Tg and HDL-C.

levels of HDL-C are components of an atherogenic lipoprotein phenotype (ALP; Austin et al. 1990*b*), and it is interesting to note that these relationships were observed both between and within normal and LpL-heterozygous subjects. The strategy of using within-genotype adjustment of LDL particle size for Tg and HDL-C removed the variance in LDL particle size related to ALP *within* genotypes and preserved the relationship of LDL particle size to ALP *between* genotypes. Thus, in families with structural mutations in the LpL gene, LpL heterozygosity accounts for only a portion of ALP. A residual relationship among LDL particle size, Tg, and HDL-C exists, both in normal and LpL-heterozygous subjects, suggesting that there are additional factors controlling this phenotype.

The present study provides strong evidence that LpL plays a primary role in lipid metabolism and the formation of small LDL particles. Low LpL activity has been shown to be associated with an increase in Tg (Nikkila et al. 1978), as a result of delayed clearance of Tg-rich LDL precursors (Taylor et al. 1980; Magill et al. 1982). This decrease in LpL activity leads to an increase in LDL particle density (Hokanson et al. 1997*b*). In addition, low LpL activity is associated with a decrease in levels of HDL-C (Nikkila et al. 1978) and apo-A-I (Magill et al. 1982) plasma levels. The present study shows that a genetic defect in the LpL gene, which leads to reduced LPL activity, influences lipid metabolism and leads to smaller LDL particles.

The structural mutations examined in this study are too rare to account fully for the common trait of small LDL particle size in the general population. However, two variants in the LpL gene, D9N and N291S, cause modest reductions of LpL lipolytic activity (Zhang et al. 1996) and are relatively common (Mailly et al. 1995; Assmann et al. 1996; Mailly et al. 1996). Given the results of the present study, it will be important to examine the role of these and other variations in the LpL gene as causes of small LDL particles. In addition, structural variants in the LpL gene also are associated with coronary disease (Hokanson 1997; Nordestgaard et al. 1997). The results reported here suggest that the increase in small LDL particles, and the associated lipid abnormalities, are a potential mechanism by which heterozygosity for LpL mutations could lead to an increase in coronary disease risk.

In conclusion, LOD score–linkage analysis provides highly statistically significant evidence for genetic linkage of LDL particle size to the LpL locus, in families with structural mutations in the LpL gene. The present study dramatically illustrates the importance of the use of a quantitative trait (i.e., LDL particle size) in linkage analyses and the value of covariate adjustments (i.e., LDL particle size adjusted for within-genotype affects of Tg and HDL-C), when mapping QTLs. In addition, this study suggests that a potential mechanism by which subjects heterozygous for structural mutations in the LpL gene are at increased risk for coronary disease is through the formation of small LDL particles.

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